Amino acid metabolism, branched-chain amino acid feeding and brain monoamine function

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Several papers in the present symposium emphasize the importance and interplay of carbohydrate and fat metabolism during exercise of varying intensities and duration. In constrast, amino acid metabolism is deemed to make only a minimal contribution to the provision of energy for the working muscle and is, with few exceptions, ignored. Equally, the mechanisms of what is termed 'central fatigue', i.e. fatigue associated with alterations in the functioning of the brain or central nervous system (CNS), are largely unexplored. However, several papers over the past few years have promoted an attractive role for certain amino acids as substrates for intermediary metabolism and precursors of brain neurotransmitters. The focus of the present paper is the potential interplay between peripheral substrate utilization, amino acid metabolism, brain neurotransmitter function and fatigue. For the purpose of the present symposium only experimental data obtained from human subjects will be considered.

Fatigue during exercise is commonly defined as the inability to maintain the required force or power output. In the present discussion, fatigue, or the perception of fatigue, is considered to be related to the loss of power output, or the perceived inability to maintain power output, during prolonged submaximal dynamic exercise. Fatigue during this type of exercise can be conveniently partitioned into 'central' and 'peripheral' components (Fig. 1).

Much is now known of the role of substrate depletion in the aetiology of fatigue (see relevant papers in the present symposium) but subjects performing prolonged exercise perceive an increase in fatigue well in advance of the point where substrate depletion (or other components of peripheral fatigue) forces the subject either to stop or to reduce the required intensity of exercise. This perception of fatigue must reside within the brain and CNS, is thought to be related to a change in brain neurotransmitters such as serotonin (5-hydroxytryptamine), noradrenaline and dopamine, and is generally known as the 'central fatigue hypothesis'. In essence, the central fatigue hypothesis suggests that an increase in brain serotoninergic function in response to an increase in the blood-borne delivery of its amino acid precursor, tryptophan, can impair certain aspects of CNS function (possibly by reducing dopaminergic activity) in order to induce an early onset of fatigue during prolonged exercise.

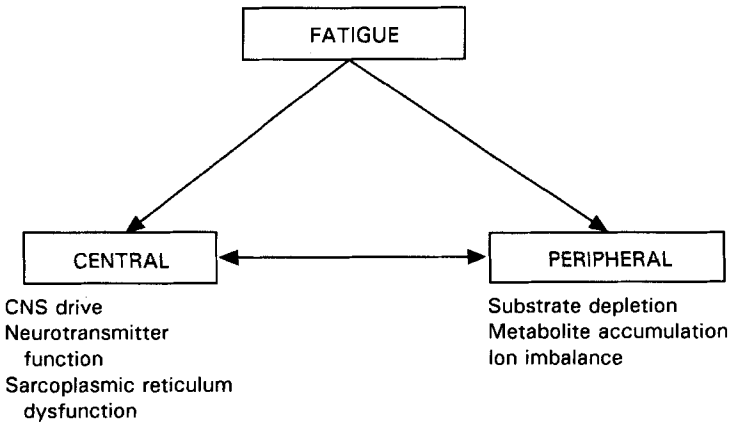

Fig. 1. Central and peripheral components of fatigue and the putative links in communication between them. CNS, central nervous system.

Abbreviations: BCAA, branched-chain amino acids; CNS, central nervous system; $K_{m(app)}^{TRP}$, apparent K_m for tryptophan; LNAA, large neutral amino acids; V_{O_2max}, maximum O_2 uptake.

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The concept of a central fatigue hypothesis probably originated from nutritional studies by Fernstrom & Wurtman (1971, 1972) and much of the interest in the relationship between nutrition, brain neurochemistry and sports performance in human subjects can be traced back to this work. In a series of papers these authors described the effect of manipulating, by dietary means, the plasma concentration of amino acids on amino acid entry into brain. Amino acids do not readily enter across the blood–brain barrier and have to be transported by means of a carrier or transporter. The large neutral amino acid (LNAA)-transporter is a saturable carrier that shares its transporter function between six amino acids: tyrosine, tryptophan, phenylalanine, and the branched-chain amino acids (BCAA) leucine, isoleucine and valine. The apparent affinity of the LNAA transporter, and hence the relative rate of transport into brain of the individual amino acid, depends on the concentration of each amino acid relative to its competitors. Of interest in the present discussion is the manipulation of the relative rate of entry of the amino acid precursors of brain monoamines, i.e. tyrosine, as a precursor for the catecholamines, and tryptophan as a precursor for serotonin (Fernstrom, 1990). Of the six competing amino acids for the LNAA transporter, tryptophan has the lowest concentration in plasma (50 μM), with approximately 90% of the tryptophan associated with plasma albumin and approximately 10% i.e. 5 μM in the free form. The plasma concentration of the competing five LNAA is approximately 10-fold higher (i.e. 500 μM), which equates to a value for free tryptophan : LNAA of 1 : 100 and an apparent K_m for tryptophan ($K_{m(app)}^{TRP}$) of 1 mM (van Hall *et al.* 1995). Studies in rats had shown that manipulation of the plasma amino acid composition to change the free typtophan : LNAA value was known to alter the rate of entry of tryptophan into brain and to increase the synthesis and subsequent metabolism of serotonin in brain (Chauloff, 1989). In the knowledge that tryptophan concentration in dietary protein is lower than that of the other amino acids competing for the LNAA transporter, Fernstrom (1983) was able to demonstrate that the intake of a protein-rich meal effectively increased $K_{m(app)}^{TRP}$ and thereby brain tryptophan concentrations remained largely unchanged, whereas feeding a high-carbohydrate diet provoked an insulin-induced clearance of several of the competing amino acids, decreased the $K_{m(app)}^{TRP}$ and promoted an influx of tryptophan into the brain. The putative behavioural effects of an increased synthesis of brain serotonin, as a result of the increase in brain typtophan, i.e. lethargy, sleep and fatigue (Young, 1986), made this an attractive proposition for those investigating the link between exercise-induced changes in peripheral amino acid metabolism, brain neurotransmitter function and fatigue, i.e. the 'central fatigue hypothesis' as proposed by Newsholme *et al.* (1987).

According to these arguments it is possible to analyse the experimental data published to date and to test the validity of the 'central fatigue hypothesis'. For clarity the experimental data are considered in three sections:

1. manipulation of the BCAA and/or tryptophan concentrations in the circulation (peripheral effect);
2. pharmacological manipulation of brain monoaminergic function (central effect);
3. cross-sectional and longitudinal training studies (genotypical and phenotypical response).

Manipulation of plasma BCAA and/or tryptophan concentrations

Several studies have attempted to manipulate the influx of tryptophan into brain and hence, according to the central fatigue hypothesis, endurance performance by nutritional intervention. The first, and most applied, study was that of Blomstrand *et al.* (1991) who investigated the effect of feeding 16 g of a mixture of BCAA on the performance of marathon runners. The BCAA cocktail was composed of valine, leucine and isoleucine in the proportions 50 : 35 : 15 in a solution containing 50 g carbohydrate/l. Oral ingestion of the BCAA drink elevated plasma BCAA levels by 140% and decreased the plasma free tryptophan : BCAA by 180%, but had no overall effect on marathon performance in the faster runners, i.e. those completing the marathon in less than 3 h 5 min, but was associated with a small improvement in performance (approximately 3%) in the slower runners. In a similar study by the same group (Newsholme *et al.* 1991), no significant change in exercise performance resulted from feeding the same BCAA cocktail to athletes competing in a 24 km event. There are obvious limitations to field-based studies, and later controlled studies in the laboratory were undertaken to verify these early results. Varnier *et al.* (1994) infused approximately 20 g BCAA or saline (9 g NaCl/l) over the 70 min period before exercise and found no difference in performance in a graded exercise test to fatigue. The conclusion from this study was to cast doubt on the validity of the central fatigue hypothesis, although it could equally be argued that the short-term incremental test used in this study was not an appropriate exercise challenge to test the central fatigue hypothesis as originally stated. Of the controlled laboratory studies in this area the paper by van Hall *et al.* (1995) is by far the most comprehensive. In this study, endurance-trained cyclists were fed on either a low (2 g/l) or high (6 g/l) concentration of BCAA in a solution containing 60 g sucrose/l, which increased the plasma BCAA concentration at exhaustion by approximately 2- and 5-fold for the low- and high-BCAA drinks respectively. Compared with a placebo, neither the low- nor high-BCAA condition had any influence on cycle performance times to exhaustion at 70% maximum O_2 uptake (V_{O_2max}). Again these findings would not appear to support the central fatigue hypothesis. However, the lack of any significant change in exercise performance following BCAA feeding is fully justified from a consideration of the underlying biochemistry relating to the process of transport of tryptophan and other competing amino acids into brain. From the experimental data it was possible to determine the effect that the change in circulating BCAA levels had on $K_{m(app)}^{TRP}$ for the amino acid transporter and thereby, from Michaelis–Menten kinetics, the change in unidirectional flux of free tryptophan into the brain (Table 1). As

mentioned previously, resting concentrations of competing LNAA ($K_{m(app)}^{TRP}$ approximately 1 M) effectively act to restrict the entry of free tryptophan into the brain (calculated unidirectional influx of 2·6 nmol/min per mg). In the control or placebo condition, prolonged exercise acts to decrease circulating BCAA levels and increase circulating free tryptophan levels, resulting in a decrease in the $K_{m(app)}^{TRP}$ and an approximate 3-fold increase in the influx of tryptophan into brain. When similar calculations are made from the experimental data obtained from the subjects following feeding of either the low- or high-BCAA drinks, the resultant changes in tryptophan influx into the brain, compared with the placebo condition, are minimal (Table 1). Considering the relatively small overall effect that any change in circulating concentrations of amino acid precursors of monoamine synthesis may have on brain monoamine function, it is totally reasonable that such small decreases in the rate of influx of free tryptophan brought about by BCAA feeding were found to have no effect on endurance performance. Furthermore, any nutritional strategy that delivers high concentrations of BCAA to muscle must take account of the increase in the production of metabolites of amino acid oxidation by muscle, i.e. glutamine and NH_3. Buffering of the NH_3 load in muscle leads to the efflux of high concentrations of glutamine, a process which can drain the muscle of intermediates of the tricarboxylic acid cycle and conceivably impair the muscle's capacity for oxidative metabolism (Wagenmakers *et al.* 1991), and thereby contribute to a peripheral cause of fatigue. Equally, efflux of NH_3 from muscle results in elevated plasma and brain NH_3 concentrations, which can lead to an impairment of motor function and/or produce symptoms of fatigue independent of any change in brain monoamine function (Banister & Cameron, 1990).

An alternative nutritional intervention that can be applied to investigate the role of amino acid precursors of brain monoamine synthesis in the aetiology of the fatigue process is to manipulate tryptophan availability directly. Feeding tryptophan elevates total tryptophan levels in the circulation, saturating the binding capacity of plasma albumin, and thereby significantly elevating the level of free tryptophan available for transport across the blood–brain barrier. The assumption in this strategy is that by increasing plasma tryptophan, and thereby tryptophan availability to the brain, the resultant increase in serotoninergic action would reduce exercise performance time. Early attempts to test this hypothesis were flawed in their experimental design by the lack of any measurement of changes in the independent variable, i.e. plasma tryptophan, and in the choice of the exercise challenge, i.e. high-intensity exercise lasting less than 10 min in duration. Paradoxically, it was found that pre-ingestion of a total of 1·2 g tryptophan (four doses of 300 mg each) produced an increase in treadmill performance time at 80 % of V_{O_2max} (Segura & Ventura, 1988) but had no effect on treadmill performance at 100 % of V_{O_2max} (Stensrud *et al.* 1992); the analgesic action of tryptophan was cited as the reason for the increase in performance in the former study. Later studies using an endurance-based exercise challenge (bicycle exercise at 70 % V_{O_2max}) in which the plasma tryptophan concentration was elevated 9-fold (by ingestion of a total of 3·9 g tryptophan) before exercise showed no effect on exercise performance when compared with a placebo (van Hall *et al.* 1995). The absence of effect on exercise performance occurred in the presence of an estimated 20-fold increase in free tryptophan influx into brain (Table 1), which seriously questions the validity of the central fatigue hypothesis to attenuate exercise performance by a mechanism which alters tryptophan availability to brain.

The opposing action, i.e. to decrease tryptophan availability and thereby reduce the influence of plasma tryptophan on the fatigue process, can be attained either by maintaining the majority of tryptophan in association with plasma albumin, therefore rendering tryptophan unavailable for transport across the blood-brain barrier, or to reduce the influence of tryptophan by removal of tryptophan from the circulation. Carbohydrate feeding has a well-known suppressive effect on the mobilization of fatty acids that compete with tryptophan for binding sites on plasma albumin and such a nutritional intervention should reduce, or negate, the increase in plasma free tryptophan

Table 1. Calculated changes in apparent K_m of tryptophan ($K_{m(app)}^{TRP}$) and unidirectional influx (v) of free tryptophan following experimental manipulation of the plasma concentration of branched-chain amino acids (BCAA) and tryptophan (Adapted from van Hall *et al.* 1995)

(Mean values and standard deviations)

		Control		Tryptophan		$BCAA_{low}$		$BCAA_{high}$	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tryptophan (μmol/l)	pre	44	7	47	6	44	5	45	10
	exh	43	7	304	61	40	6	38	7
BCAA (μmol/l)	pre	443	92	471	35	521	160	450	54
	exh	380	42	372	22	947	186	2397	483
$K_{m(app)}^{TRP}$ (μmol/l)	pre	1008		1073		1087		1003	
	exh	935		862		1286		1286	
v free tryptophan (nmol/min per mg)	pre	2·6		2·7		2·6		2·6	
	exh	7·6		54·0		7·0		6·6	

pre, pre-exercise; exh, at exhaustion; $BCAA_{low}$, 2 g BCAA/l; $BCAA_{high}$, 6 g BCAA/l.

levels during prolonged exercise. Davis *et al.* (1992) tested the hypothesis that carbohydrate feeding would minimize the central component of the fatigue process and thereby prolong endurance, by feeding subjects 5 ml/kg per h of a carbohydrate–electrolyte solution. This strategy suppressed the normal rise (5–7-fold) in plasma free tryptophan levels during prolonged exercise at 70 % V_{O_2max} and increased overall performance time by approximately 1 h. Although it was not possible to partition the beneficial effects of carbohydrate feeding on central *v.* peripheral mechanisms of fatigue, the substantial increase in endurance performance could not be explained by changes in physiological indices of peripheral muscle fatigue, and provided a measure of support to the central fatigue hypothesis. Other nutritional strategies designed to achieve the same effect include previous feeding of an amino acid mix deplete of tryptophan designed to significantly reduce (>80 %) plasma tryptophan availability (Young *et al.* 1985), but exercise trials using this experimental paradigm have not yet been completed successfully (E Venning and PM Jakeman, unpublished results).

Pharmacological manipulation of brain monoaminergic function

Many authors would argue that the evidence presented in the previous section does not consistently support the central fatigue hypothesis, and indeed the balance of the experimental data from studies using nutritional strategies would side against the existence of any influence of altered serotoninergic action in response to changes in the delivery of blood-borne tryptophan to the brain. Few, however, would argue against the influence that pharmacologically-induced alterations in serotoninergic function have on endurance performance in the rat (for example, see Bailey *et al.* 1993). But what evidence is there that similar effects are seen in human studies?

Recent advances in the pharmacology of drugs used to probe specific neurotransmitter action in human subjects have provided the exercise scientist with an alternative approach to investigate the role of the brain monoaminergic function in the aetiology of fatigue. However, several difficulties are encountered when performing these studies. These include:

non-selectivity of drug action;
differential actions of drugs when used as an acute, single-dose mode compared with the action of the drug when given repeatedly or after a short course of treatment;
inter-individual sensitivity of subject response to a fixed amount of drug;
the inability to measure, and therefore control for, the degree of agonist or antagonist action, i.e. poor control of the independent variable.

Recognizing these limitations, pharmacological agonists and antagonists of brain serotoninergic function have been used in human studies of exercise performance and to investigate the adaptation of brain serotoninergic function to exercise training (Table 2). In general, the actions of these drugs have been shown to be specific to central (brain) neurotransmitter function with no change in peripheral neurotransmitter action, as indicated by normal cardio-respiratory and metabolic responses to exercise in the drug trial. Wilson & Maughan (1992) were the first to study the effects of a single oral dose of a serotoninergic agonist (20 mg Paroxetine) on bicycle exercise time to exhaustion at 70 % V_{O_2max} in well-trained subjects. The median exercise time to exhaustion following a single dose of paroxetine was found to be 19 % less ($P = 0{\cdot}036$) than with a placebo, which the authors conclude is evidence of a component to fatigue mediated by an increase in the activity of serotoninergic neurones in brain. It must be recognized, however, that although the drug is known to increase serotoninergic action in the raphe nuclei of the brain, projection areas from the raphe nuclei tend to exhibit reduced serotoninergic activity following acute application of this type of drug. The complexity of these drug interactions may explain, in part, why later studies have not been able to fully corroborate these early findings. Using the same exercise protocol and a generically-similar drug (70 mg Fluoxetine), Davis *et al.* (1993) reported a slight (9 %), but not statistically different, decrease in cycle performance time, whilst Jakeman *et al.* (1994*a*) reported no change in treadmill endurance time at 60 % V_{O_2max} in response to a generically-different serotoninergic agonist, D-Fenfluramine (30 mg, oral). Using the opposite approach to antagonize brain serotoninergic action, Pannier *et al.* (1995) studied the effects of a single dose of the anti-migrane agent Pizotifen, a known serotoninergic antagonist, on treadmill endurance performance at 70 % V_{O_2max}.

Table 2. Studies of the effect of pharmacological manipulation of brain serotoninergic function on human endurance exercise performance (Mean values and standard deviations)

		Endurance time (min)				
		Placebo		Drug		Statistical significance of difference between groups: *P*
Reference	Regimen	Mean	SD	Mean	SD	
Wilson & Maughan (1992)	20 mg Paroxetine, cycle at 70 % V_{O_2max}	116	(86–133)*	94	(84–127)*	0·036
Davis *et al.* (1993)	70 mg Fluoxetine, cycle at 70 % V_{O_2max}	126	8·1	115	9·0	< 0·05
Hawthorne & Jakeman (1995)	30 mg Fenfluramine, treadmill at 60 % V_{O_2max}	134	41	135	31	0·89
Pannier *et al.* (1995)	1 mg Pizotifen, treadmill at 70 % V_{O_2max}	119·8	12·5	109·4	6·7	> 0·05

V_{O_2max}, maximum O_2 uptake.
* Mean values and ranges shown in parentheses.

Compared with a placebo, endurance time in the anti-serotoninergic trial decreased ($P > 0.05$) in seven of the eight subjects in this study. The data on human subjects, therefore, are inconclusive, although, since the use of pharmacological agents as 'probes' of brain neurotransmitter function and in the investigation of the 'central' component has yet to be fully established, there are exciting prospects for their future use.

Cross-sectional and longitudinal training studies

It is intriguing to know whether the lower perception of effort reported by endurance-trained athletes when compared with non-trained subjects exercising at the same absolute and/or relative exercise intensity could be accounted for by changes in peripheral amino acid metabolism, brain monoamine function and the central fatigue hypothesis as outlined in the previous two sections. In relation to the peripheral metabolism and the availability of amino acid precursors of brain monoamines, it is somewhat of a paradox that the increase in fat oxidation in the endurance-trained athletes occurs with an overall reduction in plasma non-esterified fatty acids (Hurley *et al.* 1989). This would suggest that the amount of tryptophan dissociated from plasma albumin (i.e. free tryptophan) would be less in the trained athlete than in non-trained subjects. Equally, endurance training leads to an elevated total muscle glycogen concentration and a sparing of muscle glycogen during submaximal exercise. This would indicate a lower requirement for amino acid oxidation by the exercising muscle to maintain tricarboxylic acid cycle intermediates, lower extraction of plasma BCAA and a higher circulating BCAA level (see pp. 36–38) in the trained athlete. Overall, therefore, it is to be expected that the ratio of competing amino acids for the L-amino acid transporter in brain would be weighted against tryptophan entry into brain; a factor that may account for the lower perception of effort in endurance-trained athletes. In a previous study it was possible to analyse the difference between highly-endurance-trained athletes and non-active controls in response to prolonged submaximal treadmill exercise to exhaustion. The data (Table 3) showed that, despite a lower concentration of circulating non-esterified fatty acids, the free tryptophan was slightly higher in the endurance-trained athletes and combined with the predicted increase in circulating BCAA levels, that the overall free tryptophan : BCAA value was not significantly different in endurance-trained subjects during prolonged submaximal exercise to exhaustion.

In the absence of any significant effect of endurance training on the plasma free tryptophan : BCAA ratio during prolonged exercise it could be hypothesized that the training effect leading to a lower perception of effort in the trained subjects is mediated by changes in the sensitivity of brain serotoninergic function. Using a pharmacological 'probe' of brain serotoninergic function (Buspirone) and an indirect neuro-endocrine marker of serotoninergic activity (prolactin) Jakeman *et al.* (1994*b*) were able to demonstrate a down-regulation of serotoninergic activity in response to a serotoninergic agonist in endurance-trained athletes compared with healthy, non-trained controls. The magnitude of the decrease in response in this cross-sectional comparison suggested a modification of central (brain) serotoninergic function, but could not distinguish whether the response was inherent to the subjects, or occurred as a result of an adaptation to prolonged endurance training. In a recent study, the same

Table 3. Comparison of plasma metabolite responses to prolonged treadmill exercise to exhaustion at 60% maximum oxygen uptake in endurance-trained and non-trained subjects (Adapted from Jakeman *et al.* 1994*a*)

(Mean values and standard deviations)

		Trained		Untrained		Statistical significance of difference between groups: *P*
		Mean	SD	Mean	SD	
Glucose (mmol/l)	pre	5·2	0·2	5·1	0·1	0·897
	exh	3·8	0·4	4·6	0·2	
NEFA (μmol/l)	pre	173	17	201	26	0·282
	exh	1007	138	1205	180	
Free tryptophan (μmol/l)	pre	4·0	0·3	3·6	0·2	0·079
	exh	7·8	0·7	6·3	0·5	
LNAA (μmol/l)	pre	508	28	473	20	0·004
	exh	510	16	449	22	
Free tryptophan : LNAA ($\times 10^3$)	pre	7·76		7·91		0·255
	exh	15·3		14·3		
Prolactin (mU/l)	pre	277	52	406	78	0·215
	exh	974	152	939	151	

pre, pre-exercise; exh, at exhaustion; NEFA, non-esterified fatty acids; LNAA, large neutral amino acids.

research group have performed a similar longitudinal study of the effect of 16 weeks of endurance training on previously non-trained subjects. Preliminary data indicate a mean decrease, approximating to 30 %, in response to a serotoninergic agonist following 16 weeks of endurance training (Jakeman *et al.* 1997). These preliminary findings would support the view that central monoaminergic function is implicated in the fatigue process and can be modified by endurance training.

Summary and conclusions

Although fatigue during prolonged exercise has traditionally been associated with peripheral factors relating to muscle metabolism, such as the depletion of muscle glycogen, more recent research has generated a renewed interest in amino acid metabolism *per se* and in the role of amino acids as precursors of brain neurotransmitter function. The concept of a 'central fatigue hypothesis' has done much to stimulate scientists to explore the functional role of the brain and CNS in the aetiology of the fatigue process. The concept has also generated a number of testable hypotheses by which it is possible to examine how the 'central' component of fatigue may act. The present review has attempted to bring together the current research in this area.

There is good reason to believe that nutritional intervention may play an important role in relation to fatigue residing within the brain and CNS. Although an exciting possibility exists that nutritional manipulation may affect brain neurochemistry and ultimately sports performance, the experimental evidence to support this claim is, as yet, equivocal. A greater understanding of amino acid metabolism and, in particular, amino acid transport, will greatly improve future experimental designs used to test the efficacy of nutritional manipulation of amino acids and their effect on the central component of the fatigue process.

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